IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Ngai et al.	Group Art Unit: 1656
Serial No. 09/597,608	Examiner: Taylor, J.
Filed: June 20, 2000	Attorney Docket No. B00-100-1
For: *Normalizing and Amplifying RNA*	

DECLARATION UNDER 37CFR1.132

We, Percy Luu, John Ngai and David Lin, declare and state as follows:

1. We are coinventors of the cited Serafini et al. (US Pat No. 6,114,152). Ngai and Lin are also coinventors of this patent application. Ngai is a Professor in the Department of Mocular and Cell Biology at the University of California, Berkeley; Lin is a post-doctoral research specialist in Ngai's laboratory; Luu is a doctoral candidate researcher in Ngai's laboratory. We are employed by the Regents of the University of California. the assignee of the subject patent application as well as the cited Serafini patent. We are knowledgeable and experienced in the field of expression profiling, particularly mRNA isolation, amplification and analysis. We have read and are familiar with the contents of the above application and the cited Eberwine (US Pat No. 5,514,545), Serafini, and Gudkov (US Pat No.5,866,327) references.

2. Eberwine does not teach or suggest normalizing mRNA or aRNA as required by the claims, and does not teach or suggest normalizing cDNA vs. aRNA synthesis as relied upon by the Action at p.5, lines 9-10. At col.6, lines 1-3, Eberwine says that differences in efficiency of cDNA synthesis and aRNA amplification will serve to *normalize the analysis* within an experiment. The Action appears to misconstrue Eberwine's use of the word "normalize" and applies it out-of-context to cDNA and aRNA molecules. No where does Eberwine talk of normalizing DNA or RNA molecules as suggested. In fact, Eberwine is not talking about normalizing any "thing." What Eberwine says is that the expression profile of several different *mRNAs* within the *aRNA* population allows interexperimental comparisons of a given mRNA

because differences in efficiency of cDNA synthesis will serve to *normalize the analysis*. Not the clearest language imaginable, but Eberwine does exemplify what he apparently means in the next two paragraphs.

In col.6, lines 4-40, Eberwine describes expression profiling of several hippocampal cells. In particular, Eberwine reports that he was able to separately classify hippocampal cells on the basis of the *ratio* of expression of specific mRNAs within the cell, wherein one class differed from others in the ratio of expression of K to Ca channel mRNAs. Eberwine cautions that this is not a quantitative measure of the amount of an individual mRNA, on a molar basis, but rather a 'self-controlled' comparison of hybridization intensities of these channels in the same aRNA population. In other words, the numbers of the ratio may not reflect molar amounts of the K and Ca channel mRNA. However, Eberwine assures, any *potential differences in amplification efficiency are normalized* by the similar autoradiographic intensities of other molecules used in the expression profiling, such as c-jun. In other words, Eberwine says that the differential expression of genes, such as K or Ca channels, in different experiments can be compared because genes that do not change, such as c-jun, can be used to normalize the signal ratios of the K or Ca channels between experiments.

The Action also cites col.7, lines 30-35 of Eberwine. Here, not only is the cited portion of Eberwine taken out-of-context, it is placed in a horribly misleading context. The Action discusses Eberwine's use of RNA and cDNA and then says that Eberwine teaches that a vast excess of driver RNA is used to hybridize all available cDNA. By manufacturing this juxtaposition, the Action would have us believe that Eberwine suggests use of a normalization protocol in his methods. Nothing is further from the truth.

Eberwine's contribution is characterizing single cells based on their mRNA component. While acknowledging his method will not yield full-length clones, Eberwine argues at length that they should be of high complexity (beginning at col.5, line18). He describes two ways he assessed complexity of his aRNA population: expression profiling and cDNA library screening (col.5, lines 26-29) and reports his results with both (col.5, line 30 - col.6, line 40 and col.6, line 41 - col.7, line 28, respectively). Thereafter, Eberwine explains how complexity is historically measured (col.7, lines 29-37), why this traditional measure of complexity reflects not only distinctiveness, but also abundance (col.7, lines 37-48) and argues that it is easier to isolate low

abundance mRNAs from a single cell (pursuant to his method) than from a tissue homogenate (prior methods)(col.7, lines 48-64). The portion of Eberwine cited by the Action is his description of how complexity is historically measured - Eberwine never does this and the whole point of his argument here is he doesn't need to – his method recovers low abundant mRNAs. Presumably, the Action's misleading implication was made through inadvertence.

No where does Eberwine suggest *normalizing molecules* - to the contrary, Eberwine says that any differential amplification is addressed by internal controls. Serafini does not remedy this defect as he does not teach normalizing RNA (the Action's statement to the contrary at p.7, line 5, is without support and inaccurate) or offer any motivation for modifying Eberwine to do so. Gudkov (US Pat No.5,866,327) does describe a conventional cDNA normalization protocol (e.g. col.11, lines 33-43) but does not suggests its use in the context of an amplification protocol such as claimed herein or as described by Serafini or Eberwine.

In our expert opinions, the cited art does not suggest to one of ordinary skill in the art to modify the protocol of Serafini or Eberwine to incorporate a normalization step as claimed. In fact, we were all well aware of the cited art prior to making the present invention, and at that time, it was certainly not obvious to any of us.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: Dec 21, 2000

Percy Luu

Date: Dec 21, 2000

Prof. John Ngai

Date: Dec 21, 2000

David Lin, Ph.D.